Immunology 2004 **112** 2–12 doi:10.1111/j.1365-2567.2004.01849.x

REVIEW ARTICLE

Interferon-γ activation of polymorphonuclear neutrophil function

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SUMMARY

As current research illuminates the dynamic interplay between the innate and acquired immune responses, the interaction and communication between these two arms has yet to be fully investigated. Polymorphonuclear neutrophils (PMNs) and interferon-γ (IFN-γ) are known critical components of innate and acquired immunity, respectively. However, recent studies have demonstrated that these two components are not entirely isolated. Treatment of PMNs with IFN-γ elicits a variety of responses depending on stimuli and environmental conditions. These responses include increased oxidative burst, differential gene expression, and induction of antigen presentation. Many of these functions have been overlooked in PMNs, which have long been classified as terminal phagocytic cells incapable of protein synthesis. As this review reports, the old definition of the PMN is in need of an update, as these cells have demonstrated their ability to mediate the transition between the innate and acquired immune responses.

Keywords activation; cytokines: interleukins; neutrophils; phagocytosis

INTRODUCTION

Although the interferons were first identified and named for their potent ability to interfere with and inhibit viral infections, it soon became apparent that they could be divided into two categories. Type I interferons, consisting of interferon α and β, have antiviral activity as a primary function. Type II interferon, consisting solely of interferon-γ (IFN-γ), has a multitude of immunoregulatory functions in addition to its antiviral effect.[1] Since its discovery, IFN-γ has been shown to be one of the most potent and pleiotrophic cytokines.

Investigations into the functions of IFN-γ have classically focused on the interactions of macrophages and $CD4^+$ T cells. The interaction of a T-cell receptor with an antigen bound to a major histocompatibility complex (MHC) molecule triggers production of IFN-γ by T cells. This IFN-γ then acts to activate macrophages, up-regulating a number of gene products and rendering macrophages additionally cytotoxic by increasing oxidative burst and the production of other oxidants such as nitric oxide. Recently, IFN-γ was shown to be produced by a number of other immune cell types, including natural killer cells (NK) and macrophages; and to regulate the functions of many of these cell types.

The majority of IFN-γ research has focused on IFN-γ's interactions with T cells, NK cells, and activated macrophages; all components of the secondary, acquired response. Research into the primary response, consisting primarily of polymorphonuclear neutrophils and other components of innate immunity, has overlooked the significance IFN-γ. This oversight is unfortunate, as IFN-γ has been shown to be a potent and critical modulator of the innate immune response.

This review will focus on the interactions of IFN-γ and the PMN. This type of interaction between innate and

Received 23 January 2004; accepted 16 February 2004.

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; BlyS, B lymphocyte stimulator; Con A, concanavalin A; FcγRI or CD64, Fc receptor I; fMLP, f-methionine-leucine-phenylalanine; GM-CSF, granulocyte–monocyte colony-stimulating factor; GRO-α, human growth-regulated oncogene; H_2O_2, hydrogen peroxide; I-TAC, interferon-inducible T-cell α chemoattractant; IP-10, interferon-γ inducible protein 10; IFN-γ, interferon-γ; IL, interleukin; LPS, lipopolysaccharide; MIP, macrophage inflammatory protein; MHC, major histocompatability complex; Map kinases, mitogen activated protein kinases; MIG, monokine induced by interferon-γ; NK cells, natural killer cells; NO, nitric oxide; iNOS, inducible nitric oxide synthase; NFκB, nuclear factor κB; PMNs, polymorphonuclear neutrophils; Stat1, signal transducer and activator of transcription 1; TNF-α, tumour necrosis factor-α.

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acquired immunity has been overlooked, in part caused by 'the obsolete concept of the neutrophil as a ''terminally differentiated, short-lived, cell devoid of transcriptional activities'' found in most biomedical textbooks'.[2] As will be demonstrated, PMNs are active, dynamic cells that respond to immunomodulators such as IFN-γ through complex changes in gene expression. This review will explore what is known of gene expression and regulation in response to IFN-γ at a molecular level as well as IFN-γ's effects on the traditional PMN functions of oxidative burst, phagocytosis, and chemotaxis. This discussion should lead to an improved understanding of the roles of both the PMN and IFN-γ in the innate immune response.

INTERFERON-γ STIMULATED SIGNAL TRANSDUCTION

The primary way in which IFN-γ acts as an immunomodulator is through regulation of gene expression. The signal transduction pathways leading from binding of IFN-γ and its receptor to subsequent activation of gene transcription has been well studied in cell types other than PMNs. These studies have established that the primary method of IFN-γ signal transduction is via a Jak-Stat tyrosine kinase dependent pathway. In this pathway, IFN-γ binding to the receptor triggers the phosphorylation and activation of Jak1, a tyrosine kinase, which then activates signal transducer and activator of transcription 1 (Stat1). Stat1 then forms functionally active homodimers that move into the nucleus and bind to specific DNA sequences.[3] New research in non-PMN cell types has indicated that IFN-γ has the ability to activate other signalling pathways as well. These other pathways, such as those involving mitogen-activated protein kinases (Map kinases), and are now being investigated.[3]

Only recently have the signalling pathways of IFN-γ in PMNs been investigated. Studies have found that resting PMNs express approximately 1000 receptor molecules that can rapidly and stably bind the IFN-γ molecule. After binding, many of these receptors are internalized leading to a subsequent drop in receptor sites on the surface.[4,5] Most studies into IFN-γ signalling pathways in PMNs have used the Fc receptor I (FcγRI) gene as a reporter system. The promoter region of the gene encoding FcγRI has been shown to contain an interferon-γ response region, to which IFN-γ-activated transcription factors bound. Using this reporter system, IFN-γ stimulated gene expression in PMNs was demonstrated to utilize the traditional Jak-Stat pathway through the activation of Stat1·[6] However, this same pathway has also been shown in PMNs to activate Stat3, which is not usually observed in IFN-γ signalling in other cell types.[7]

McDonald *et al*. recently compared the signal transduction pathways of a number of pro-inflammatory cytokines during their activation of FcγRI gene expression in PMNs. This study revealed that these pro-inflammatory cytokines used different transduction pathways to activate expression of the same gene. IFN-γ was shown to use the traditional Jak-Stat1 pathway, while granulocyte–monocyte colony-stimulating factor (GM-CSF) activated Stat5 activity. Tumour necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) were both shown to activate nuclear factor κB (NFκB).[8] While IFN-γ utilized a pathway common to a number of cell types, the signalling pathway of GM-CSF through Stat5 had not been previously observed. Therefore, these data indicate that the combination of one cytokine with a specific cell type can result in a unique pattern of gene expression that is triggered by the signal transduction pathway used.

Signalling pathways that utilize Ca^{2+} flux have recently been shown to be activated in IFN-γ treated PMNs. Simple treatment of PMNs with IFN-γ for short periods elicited a modest Ca^{2+} signal, and triggered increased sequestration of Ca^{2+} into intracellular compartments.[9,10] This initial response is thought to be involved in the priming of PMNs for a subsequent response. The increased Ca^{2+} sequestration was shown to prime cellular sensitivity to stimuli, allowing for a subsequent enhancement the next time that signalling pathway is used.[10] This modest initial signal has also been shown to be enhanced when IFN-γ treatment is coupled with other stimuli, such as the binding of fibronectin to PMNs.[11] Fibronectin, an extracellular matrix protein, would most likely bind to PMNs as they extravasate into the tissues, where full PMN activity would be warranted. These data indicate that IFN-γ may act via a number of signalling pathways to prepare PMNs for subsequent functions and to initiate them.

IFN-γ signalling in PMNs has been shown to use an unusual variety of signalling cascades. Single treatment of these cells with IFN-γ demonstrated that a G-protein dependent pathway was used to elicit Ca^{2+} flux[12] as increased levels of inositol triphosphate were observed.[13] However, tyrosine-kinase dependent mechanisms may also be involved, as treatment with genstein, a tyrosine kinase inhibitor, was shown to inhibit Ca^{2+} flux.[12] Increased protein kinase C activity occurred during this Ca^{2+} flux[14] and costimulation with IFN-γ and fibronectin resulted in the activation of sphingosine kinases.[11] Sphingosine kinases have not been observed to be activated by IFN-γ in other cell types. Thus, further research into signalling in this cell type is needed to resolve these preliminary findings.

INTERFERON-γ REGULATION OF GENE EXPRESSION

Historically, PMNs have not been considered capable of responding to stimuli via gene expression and protein synthesis. It was thought that PMNs reacted entirely via the secretion of preformed proteins contained within the cytoplasm and granules at the time of cell migration from the bone marrow into the blood stream. This persistent idea has been disproved repeatedly by research on PMNs. Studies into the PMN response to IFN-γ demonstrate a range of gene products whose expression is modulated by signals sensed in the environment. Table 1 lists the gene products that have been demonstrated to have their expression regulated when PMNs respond to IFN-γ. Most of the genes whose regulation was explored are functionally tied to the immune system. Many of these genes are

Table 1. Gene products induced, up- or down-regulated by IFN-γ in PMNs

Gene product	Function	Reference
Gene products induced or up-regulated by PMNs		
BLyS	B Lymphocyte stimulator	34
C3b	Complement receptor	42,48
CCL20	Dendritic chemotactic factor	119
CCR1	Chemokine receptor	44
CCR3	Chemokine receptor	44
CCR6	Chemokine receptor	22
CD11α	β2 Integrin/Adhesion	43
CD11β	β2 Integrin/Adhesion	42
CD14	LPS binding	46,47
CD18	β2 Integrin/Adhesion	42,43
CD69	Activation marker	49
CD80	Antigen presentation	18,19,23
CD83	Dendritic cell marker	19,22,23
CD86	Antigen presentation	18,19,23
gp91-phox	NADPH oxidase subunit	70
FcγRI	Antibody Fc receptor	71
FcγRIII	Antibody Fc Receptor	120
IL-1β	Pro-inflammatory cytokine	27
IL-1Ra	IL-1 receptor antagonist	121
IL-6	Anti-inflammatory cytokine	33
IP-10	IFN-γ inducible protein/chemokine	31,32
I-TAC	T cell chemoattractant	31
MIG	IFN-γ induced monokine	31
MHC II	Antigen presentation	16–20,23
PAF-acether	Platelet activating factor	122
TNF-α	Pro-inflammatory cytokine	27
Gene products down-regulated by IFN-γ in PMNs		
CXCR4	Chemokine receptor	45
GROα	Chemokine/human KC	30
IL-8	Neutrophil chemotactic factor	25,26,28
MIP-1α	Macrophage inflammatory protein	28
MIP-1β	Macrophage inflammatory protein	28
P47-phox	NADPH oxidase subunit	70,71

cytokines or chemokines, indicating that PMNs may play a critical role in signalling and directing other components of the immune response. This list of genes is currently quite limited, as most of these studies occurred prior to global gene expression technology. Recently, gene expression in LPS-treated neutrophils was more fully explored via microarray analysis. These data indicate that in addition to a number of cytokine and oxidative burst-related genes, other genes related to cell growth, cytoskeletal rearrangement, and metabolism are also differentially regulated during the response to LPS.[15] Hopefully, as more studies of this kind are performed, the idea of PMNs as cells with a limited functionality will be replaced with the more accurate view that these cells are crucial to signalling and directing a dynamic immune response.

MHC II EXPRESSION

One of the most unlikely gene products to be induced by IFN-γ in PMNs is MHC II. PMNs have traditionally been considered to be cells solely involved in innate immunity, with no function in antigen presentation or T-cell activation. *In vitro* experiments with PMNs may be casting doubt on this assumption, as a number of investigators have now reported that PMNs express MHC II on the cell surface when stimulated with IFN-γ or other pro-inflammatory stimuli, such as GM-CSF.[16–18] CD80 and CD86, costimulatory molecules required for T-cell activation, have also been shown to be up-regulated under the same conditions as induce MHC II expression.[18,19] These expressed MHC II molecules have even been shown to be at least partly functional, as the PMNs have been demonstrated to act as required accessory cells during T-cell activation with staphylococcal enterotoxin, a superantigen that does not require intracellular processing prior to presentation.[17] MHC II-expressing PMNs have also been shown to produce interleukin (IL)-8 when stimulated with this same superantigen.[20] The ability to fully process and present more fully processed antigens, such as tetanus toxoid, remains controversial. Fanger *et al.* in a side-by-side comparison of superantigen and tetanus toxoid, found the MHC II-expressing PMNs were only able to effectively present superantigen, and not tetanus toxoid.[17] However, Radsak *et al.* in a later study, were able to induce a low but statistically significant level of activated T cells in response to MHC II-expressing PMNs and tetanus toxoid.[18] One explanation for these conflicting results could be a result of genetic polymorphisms in the human population. Reinisch *et al.* in a study of 55 human subjects, found that only 51% had PMNs that would express MHCII when stimulated.[21] This donor-dependence is one possible explanation for the discrepancy in results seen with regard to antigen presentation studies. Regardless, these studies indicate that the idea of PMNs being terminally differentiated cells may need to be reinvestigated as these data indicate an ability to induce previously unknown cell functions.

Further studies of surface marker expression present additional evidence for the ability of PMNs to differentiate after leaving the bone marrow. CD83, a traditional dendritic cell marker, was shown to be expressed on the surface of PMNs stimulated with IFN-γ.[22] Further studies of these cells stimulated *in vitro* have shown that they had altered morphology, lost traditional PMN chemotactic responses, and presented antigen via MHC II; yet maintained phagocytic and oxidative burst capabilities.[23] A survey of patients with acute bacterial infections found that over half of the patients tested had circulating PMNs expressing CD83.[19] These data indicate that this phenomenon was not simply the result of unlikely *in vitro* cytokine cocktails, but demonstrated a new functionality for PMNs that should be further explored.

CYTOKINE AND CHEMOKINE EXPRESSION

Neutrophils are usually the first cell type of the immune system to arrive at a site of infection. As such, these cells are critical components of both inflammatory and antimicrobial processes. Both of these processes are tightly regulated through the production of specific cytokines. As the non-phagocytic functions of PMNs have been more fully

explored, the crucial ability of PMNs to secrete cytokines and chemokines has been demonstrated clearly. The array of cytokines and chemokines that PMNs are capable of producing has been reviewed by Cassatella.[24]

The subsets of cytokines and chemokines whose expression in PMNs is modulated by IFN-γ is shown in Table 1. It is interesting to note that IFN-γ treatment of PMNs has been shown to down-regulate IL-8.[25,26] IL-8 is a potent chemoattractant of PMNs, indicating that IFN-γ may act as a signal to halt PMN recruitment and infiltration. However, this down-regulation of IL-8 was correlated with an up-regulation of the pro-inflammatory cytokines TNF-α and IL-1β. *In vitro* experiments have shown that PMNs incubated with IFN-γ demonstrate a transient down-regulation of IL-8 for the first few hours of incubation. Extended incubation of PMNs with IFN-γ leads to production of TNF-α and IL-1β. This new cytokine milieu then acts in an autocrine manner to override the signal from IFN-γ and reactivate IL-8 synthesis.[27] A similar pattern of changes in the cytokine network resulting in reactivated gene expression by PMNs was observed with macrophage inflammatory protein (MIP)-1α and MIP-1β expression.[28] Given these observations, it appears that PMN responses may change over time as the cytokine milieu changes, and that cytokine production by PMNs may act as much on themselves as on other cells and cell types.

Many of the other signalling molecules produced by IFN-γ-stimulated PMNs are chemokines. It should be noted that IFN-γ appears to down-regulate those chemokines that recruit neutrophils, and up-regulate chemokines that are chemoattractants for components of the acquired immune response, specifically T cells. PMN-derived MIP-1α, MIP-1β, and human growth-regulated oncogene (GRO-α)/murine keratinocyte-derived chemokine were shown to be down-regulated by IFN-γ treatment.[28–30] These three chemokines all recruit phagocytic cells such as neutrophils and macrophages. Up-regulated chemokines include IFN-γ inducible protein 10 (IP-10), monokine induced by IFN-γ (MIG), and IFN-inducible T cell α chemoattractant (I-TAC),[31,32] all specific for activated T cells. Additionally, IL-6, a molecule thought to be involved in the transition from an innate to an acquired response, and B lymphocyte stimulator (BLyS), a pro-B-cell factor, have also been shown to be up-regulated.[33,34] These data all indicate that IFN-γ stimulates PMNs to signal other components of the immune system.

IFN-γ has typically been considered to be secreted by and to act on components of the acquire immune system, such as macrophages or T cells. As this review demonstrates, the actions of this potent cytokine are not limited to these cell types but can have dramatic effects on PMNs. Recent research indicated that PMNs may be an important source of this cytokine. *In vitro* experiments demonstrated that human PMNs stimulated with a combination of LPS, IL-12, and TNF-α secrete low levels of IFN-γ.[35] Peritoneal murine PMNs have also been shown to express IFN-γ mRNA *in vitro* after stimulation with LPS.[36] *In vivo* experiments have revealed that PMNs secrete IFN-γ in response to a variety of infectious agents including *Nocardia asteroides*,[37] *Salmonella typhimurium*,[38] *Leishmania major*[39,40] and *Plasmodium berhei*.[41] During pulmonary infection with *Nocardia asteroides*, PMNs were found to be the sole source of IFN-γ during the course of infection[37] and this response depended on both the viability and growth stage of the organism (unpublished observations). PMN-derived IFN-γ was also found to be required for macrophage control of leishmanial growth and for stimulation of $CD4^+$ T cell migration and cytokine production. In this system, IFN-γ production involved PMN binding of macrophage surface CD28, a molecule usually thought to be involved in T-cell stimulation.[40] Further investigations into the role of PMNs as modulators of the immune response are needed to elucidate the frequency of a PMN IFN-γ response and the importance of PMNs as sources of this powerful cytokine.

OTHER SURFACE MARKERS

In addition to the surface markers already discussed, IFN-γ regulates the expression of a number of other receptors and integrins on the PMN cell surface. Many of those up-regulated are related to PMN adherence and extravasion, such as the integrins CD11α, CD11β and CD18.[42,43] Chemokine receptors CCR1, CCR3 and CCR6 are also up-regulated[22,44] while CXCR4 is down-regulated[45] indicating that IFN-γ may be coupled with other signalling molecules to co-ordinate specific recruitment of cells to the site of infection. Other markers whose expression is enhanced indicate that IFN-γ usually acts as an activating agent for PMNs. These molecules include CD14, the primary binding site for bacterial LPS[46,47] and the complement component C3b.[48] Recently, IFN-γ treatment was shown to induce CD69 expression on PMNs.[49] CD69 is known as an early activation marker for B and T cells. These data suggest that CD69 can be used as a more general marker of activation. Expression of CD69 was observed to correlate with PMN production of IFN-γ (unpublished observations). Interestingly, IFN-γ acts to down-regulate the expression of the IFN-γ receptor on the surface of PMNs.[4,5] Given the demonstrated time-dependent nature of the response of PMNs to IFN-γ, this may be a regulatory mechanism used to prevent PMNs from causing damage during the resolution of infection and inflammation.

PRIMING OF NEUTROPHIL FUNCTIONS BY IFN-γ

While recent investigations demonstrated signal transduction and gene expression in IFN-γ-treated PMNs, most studies have focused on PMN function at a cellular level. Specifically, these studies targeted IFN-γ's action as a priming agent. The term 'priming' refers to a stimulus that prepares PMNs for enhanced activity upon secondary stimulation. A variety of traditional PMN functions may be primed, including increased oxidative metabolism, surface receptor expression, degranulation and other functions associated with traditional PMN activities.[50] The priming

effect of IFN-γ on PMNs include, but is not limited to, these traditional PMN functions. A number of other cytokines have also been shown to act as priming agents for PMNs, including TNF-α and IL-8.[51] However, these responses were demonstrated to vary with both the cytokine and the second stimulus involved. A full investigation into the combinatorial effects of priming agent and stimulus could uncover the methods of this finely tuned control of the PMN response.

OXIDATIVE BURST

Traditionally, the oxidative burst, or production of reactive oxygen species, has been considered to be a PMN's primary and most important function. With the aim of destroying the foreign invaders, PMNs infiltrate a site of inflammation or infection where they are stimulated to release reactive oxygen species. Hydrogen peroxide (H_2O_2) and superoxide (O_2^-) are the primary reactive oxygen species produced during oxidative burst. These species can react with other chemical components to create reactive halides, hydroxyl radicals (OH^-), and singlet oxygen.[52,53] The release of these reactive and toxic compounds results in damage to the targeted cell or object, and often results in irreparable damage and death to the PMN itself.

A multitude of signals, both foreign and host cell derived, have been demonstrated to stimulate oxygen metabolism and oxidative burst. While not considered a traditional PMN activator, IFN-γ has been demonstrated to enhance, or prime, increased reactive oxygen species production in combination with a secondary stimulus.[54,55] Berton *et al.* were the first to observe the priming effect of IFN-γ on PMN oxidative burst. When pretreated with IFN-γ, PMNs stimulated with either f-methionine-leucine-phenylalanine (fMLP), concanavalin A (Con A), or LPS demonstrated increased O_2 consumption and O_2^- production.[56] A number of later studies confirmed these observations, documenting increased H_2O_2 production, O_2^- production, and increased reducing power in response to a variety of chemical stimuli including fMLP, Con A, LPS, and zymosan.[42,57–62] Interestingly, IFN-γ did not enhance the PMN response to the stimulus phorbol 12-myristate acetate, which stimulates cells without the use of a surface membrane receptor.[57,63,64] These results indicated that the IFN-γ priming effect may be specific to stimuli that act via membrane associated receptors. The IFN-γ priming response was demonstrated in a number of species including humans,[56] cows,[62] mice[65] and rats.[66]

The IFN-γ priming effect was found to be dose and time dependent. Doses as low as 2 U/ml enhanced production while doses ranging from 50 to 1000 U/ml elicited an optimal response, dependent on the incubation period.[58,64] While preincubation of PMNs with IFN-γ for a period as short as 10 min was shown to enhance oxidative burst[60,67] incubation for an hour or longer was shown to have a maximal effect on oxidative burst.[56,68] Incubation of IFN-γ-treated PMNs with protein synthesis inhibitors such as cyclohexamide (an inhibitor of translation), or actinomycin D (an inhibitor of RNA synthesis), inhibited oxidative burst. These results indicate that the enhancement of oxidative burst by PMNs is dependent on new protein synthesis.[56,58,64]

The primary enzyme involved in the production of reactive oxygen species is reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. NADPH oxidase is a multiunit enzyme complex that fully assembles upon cellular stimulation to catalyze the formation of superoxide anion. The main regulated subunit of NADPH oxidase is the membrane protein gp91-phox.[55] Mutations in this gene were correlated with chronic granulomatous disease.[55] IFN-γ treatment was demonstrated to up regulate the expression of the gp91-phox subunit in a protein synthesis dependent manner.[69,70] However, the p47-phox subunit, which is constitutively expressed in resting PMNs, was shown to be down-regulated by IFN-γ exposure.[71] While the regulation of these subunits remains a complex process that is not fully understood, the end result of IFN-γ treatment has been clearly demonstrated to be priming of the oxidative response.

NITRIC OXIDE PRODUCTION

The production of nitric oxide (NO) during the oxidative burst of PMNs has been a subject of some debate. Inducible nitric oxide synthase (iNOS), the enzyme involved, had only recently been observed to be expressed by PMNs of any species. Inducible nitric oxide synthase expression and NO production were shown to be induced by IFN-γ. McCall *et al.* first observed that iNOS expression in rat PMNs was enhanced in a cyclohexamide-dependent manner by IFN-γ.[72] This observation has since been extended to human PMNs, with the demonstration that iNOS expression is IFN-γ dose dependent[73] and that the gene product colocalizes with myeloperoxidase in the primary granules.[74] This colocalization suggests that iNOS is directly involved with the enhanced oxidative burst and cytotoxicity of IFN-γ treated PMNs, as myeloperoxidase catalyses the reaction of H_2O_2 into more toxic intermediates. This colocalization of these two enzymes may result in enhanced reaction of H_2O_2 with NO to form the highly reactive and toxic peroxynitrite ($ONOO^-$) molecule.

PHAGOCYTOSIS AND CYTOCIDAL EFFECTS

Treatment of PMNs with IFN-γ was demonstrated to have significant effects on the functions of phagocytosis and cell killing. Short-term treatment of PMNs with IFN-γ (20–30 min) was shown by Shalaby *et al.* to increase the phagocytosis of latex beads[75] and other studies demonstrated increased phagocytosis of *Plasmodium falciparum* merozoites induced by IFN-γ treatment.[76] Studies with IFN-γ knockout mice have also demonstrated that PMNs from these mice exhibit a twofold reduction in phagocytosis. Because the primary function of PMNs is to damage and destroy foreign microbes, numerous studies have investigated how IFN-γ treatments modulate this ability. IFN-γ has been shown to be a potent stimulator of cytocidal activity, as shown in Table 2.

Table 2. IFN-γ treatment enhances PMN killing of the following organisms

Organism	Reference
Bacteria	
Brucella abortus	90
Enterococcus faecalis	92
Legionella pneumophila	89
Mycobacterium fortuitum	91
Mycobacterium tuberculosis	93
Staphylococcus aureus	67
Fungi	
Aspergillus fumigatus	88
Blastomyces dermatitidis	81–83
Candida albicans	59,65,77–80
Candida parasilosis	80
Candida tropicalis	80
Paracoccdioides brasiliensis	85–87
Pencililium marneffi	84
Protozoa	
Entamoeba histolytica	94
Plasmodium falciparum	95
Other	
Gastric endothelial cells	63
Litomosoides sigmodontis (filaria worm)	123
Tumour cells	73,96,124

The effect of IFN-γ on the PMN response to a variety of fungi has been thoroughly investigated. In general these studies found some form of enhanced anti-fungal activity from PMNs treated with IFN-γ. Studies of the PMN response to *Candida* revealed an IFN-γ dose dependent inhibition of growth, hyphal damage, or hyphal killing with both human peripheral blood and mouse peritoneal PMNs.[65,77–80] Studies with *Blastomyces dermatitidis* have shown an enhanced oxidative burst within the first 6 hr post-treatment with IFN-γ that changed the effect of PMN attack from fungistatic to fungicidal.[81–83] This shift from a fungistatic to a fungicidal effect with IFN-γ treatment was further demonstrated with *Penicillium marneffei*[84] and *Paracoccidioides brasiliensis*.[85–87] Increased oxidative burst was also observed in response to *Aspergillus fumigatus*. This IFN-γ triggered response was shown to be to be dependent on new transcription and protein synthesis.[88]

IFN-γ has also been demonstrated to enhance the bactericidal activity of PMNs towards a variety of bacterial species, including *Brucella abortus*, *Legionellla pneumophila*, *Enterococcus faecalis* and *Mycobacterium fortuitum*.[89–92] However, not all species investigated used oxidative burst to enhance bactericidal activity. Responses to *Brucella abortus* and *Enterococcus* were demonstrated to be the result of increased superoxide anion or hydrogen peroxide secretion.[90,92] However, the bactericidal response to *Mycobacterium fortuitum* was observed to result from nonoxidative mechanisms.[91] This non-oxidative response to *Mycobacterium fortuitum* coincided with an increased time to killing (18 hr) as compared to those studies linked to oxidative burst. The effect of IFN-γ on PMN responses to *Mycobacterium tuberculosis* has also been investigated, with IFN-γ being observed to inhibit the bactericidal activity of PMNs.[93] These data indicate that IFN-γ-primed responses to different bacterial species vary widely and may be the result of PMN recognition of species-specific surface molecules.

IFN-γ-primed PMN killing of non-fungal eukaryotic targets has also been observed. IFN-γ treatment was shown to enhance contact-dependent killing of *Entamoeba histolytica*, with production of hydrogen peroxide shown to be required.[94] IFN-γ-treated PMNs have also been demonstrated to inhibit the growth of *Plasmodium falciparum* and to kill the parasite via a phagocytic mechanism.[95] IFN-γ-primed PMNs have also been shown to effect the killing of tumour cells. Interestingly, the cytocidal effect of these PMNs was found to be bimodal, with an initial (5 min post incubation), trypsin-sensitive cytocidal mechanism, followed after 180 min of IFN-γ incubation by a second cytocidal mechanism that is trypsin-insensitive.[96] This two-phase effect may be similar to or linked with to the bimodal regulation of IL-8 expression described earlier. The changing stimulatory environment and cytokine milieu may result in changes of PMN functions over time. These studies of cellular killing mechanisms of IFN-γ primed PMNs suggest that PMNs exhibit multiple mechanisms of killing elicited by differences in the target cell components.

FC RECEPTOR EXPRESSION AND ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC)

ADCC was shown to be enhanced by IFN-γ treatment of PMNs. This effect was demonstrated in both human and bovine cells to require a 2–4 hr incubation period with IFN-γ.[75,97,98] However, ADCC did not require RNA or protein synthesis. Using bovine PMNs, Steinbeck *et al.* demonstrated that IFN-γ enhanced ADCC of chicken erythrocytes even in the presence of RNA and protein synthesis inhibitors.[99] Non-specific cell cytotoxicity, in which IgG activated PMNs kill non-opsonized bystander cells, was not affected by IFN-γ treatment, although IgG treatment stimulated increased production of superoxide and hydrogen peroxide.[100]

Enhanced ADCC in IFN-γ-primed PMNs was closely correlated with expression of FcγRI by the PMN.[101] The high affinity receptor for monomeric IgG1, FcγRI (CD64), is not found on the surface of resting PMNs. FcγRII, the receptor for polymeric IgG1 and IgG2, and FcγRIII, both low-affinity receptors for various forms of IgG, are expressed constitutively at low levels on resting PMNs.[102] Fc receptor I (FcγRI), but not FcγRII or FcγRIII, was shown to be induced in both human and bovine PMNs by IFN-γ treatment.[101,103–105] This induction required an extended exposure time to IFN-γ, as treatments for less than 1 hr have no effect on *Fc*γRI expression.[106] Treatment with 100 U/ml IFN-γ for 4–5 hr was shown by Hoffmeyer *et al.* to induce an average 15 000 FcγRI molecules on the surface of each PMN[107] with mRNA of FcγRI induced by IFN-γ 1 hr post treatment.[108] FcγRI expression has been correlated with increased ADCC,[102,104,109] increased

microbicidal activity[110] and increased oxidative burst via the activation of NADPH oxidase.[107] At the molecular level, the binding of neutrophil FcγRI to IgG1 was shown to activate similar signal transduction pathways as seen on monocytic cells, including Ca^{2+} flux, and required tyrosine kinase activation.[107] However, the expression of FcγRI on PMNs, unlike on monocytic cells was inhibited by the immunosuppresant dexamethazone[111] and was not affected by inhibitors of Na^{+}/H^{+} antiporters, as was observed in monocytic cells.[108] These data, like those seen during investigations into Ca^{2+} flux signalling, indicate that PMNs may use novel pathways of signalling to control gene expression, and to elicit the appropriate response.

CHEMOTAXIS AND APOPTOSIS

IFN-γ has never been demonstrated to have a chemotactic effect on PMNs. In the human, bovine and murine systems, IFN-γ was found not to have a chemotactic effect on PMNs, but rather inhibited both random and directed migration.[112–114] This suppression of cellular migration was observed both in *in vitro* experiments and *in vivo* in the mouse peritoneal cavity.[113] Additionally, IFN-γ suppressed the chemotactic migration of PMNs toward fMLP. This inhibitory effect was demonstrated to be independent of protein synthesis or tyrosine kinase activity.[114] *In vitro*, IFN-γ increased the rate of PMN adherence.[115] These data indicate that IFN-γ may act as a signal of arrival to a site of inflammation allowing for the accumulation of PMNs.

PMNs, as terminally differentiated cells, are short-lived and readily undergo apoptosis, or programmed cell death. Stimulation of PMNs with a number of activating substances, including IFN-γ, has been shown to extend the life span and functional activity of these effector cells. Colotta *et al*. found that IFN-γ treatment reduced the number of PMNs with apoptotic morphology by 10-fold after 48 hr in culture, and that the *in vitro* life span of PMNs could be extended from 48 hr to beyond 96 hr via IFN-γ treatment.[116] This extension of the lifespan of PMNs has also been observed *in vivo* in cells from bacterial sepsis patients.[117] Suppression of apoptosis was also correlated with other functions induced by IFN-γ, such as FcγRI expression and enhanced oxidative burst.[107,118] Similarly to what has been reported in other cell types, suppression of apoptosis in PMNs involved tyrosine-kinase dependent pathways.[117]

CONCLUSIONS

PMNs are the first cells to respond to an infection. As such it is logical to think of these cells as evaluators of the scene at hand, dynamically responding to the environmental conditions and directing the subsequent response of other immune cell types such as T cells, B cells, and macrophages. Research on the non-phagocytic roles of PMNs has been hampered by both the persistent belief that PMNs have a limited range of functionality and difficulties in culturing this cell type for *in vitro* experimentation. However, as we understand better the crosstalk and integrated nature of the innate and acquired immune responses, the 'non-traditional' role of PMNs to respond to stimuli via gene expression and secretion may prove to be of critical importance. Indeed, the data discussed in this review demonstrate that this is the case, as IFN-γ-induced gene expression has been seen to be critical for enhanced ADCC and effective cytotoxic mechanisms against a wide variety of microbial pathogens. The production of IFN-γ, and other cytokines, by PMNs clearly illustrates the ability of these cells to stimulate and direct an appropriate immune response. Even though IFN-γ and PMNs might seem an unlikely combination, this review shows it is an important and dynamic one.

ACKNOWLEDGMENTS

Public Health Service grant R01-HL69426 from the National Heart, Lung and Blood Institute funded this work.

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